Acoustic Surveying of Subterranean Insect Populations in Citrus Groves

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ABSTRACT Subterranean insect pests cause considerable economic damage but their concealment makes detection difficult. A portable acoustic system was developed and tested for its potential to rate the likelihood that trees in citrus groves were infested with Diaprepes abbreviatus (L.) larvae. The likelihood was rated independently by a computer program and an experienced listener that distinguished insect sounds from background noises. Diaprepes abbreviatus, Phyllophaga spp., or other pest insects were excavated from all 11 sites rated at high likelihood of infestation but were absent from 20 of 25 low-rated sites. There was a significant regression between the activity rate and the number of pest organisms present at recording sites although the correlation was weaker than between activity rate and likelihood of infestation. Although the system is at an early stage of development, the success of these field tests suggests that it has considerable potential as a tool to detect and monitor hidden infestations of insects in soil.

KEY WORDS Phyllophaga, Diaprepes, detection, grubs

Nondestructive, rapid survey methods are needed to detect economically important subterranean insect pests, including the Diaprepes root weevil, Diaprepes abbreviatus (L.), and Phyllophaga spp. (Diaprepes Task Force 1995, Crocker et al. 1996, Simpson et al. 1996). Neonate D. abbreviatus larvae feed on the small fibrous roots of citrus and ornamental trees, and later instars feed also on lateral roots and the crown of the root systems (Beavers and Selhime 1975). This reduces the trees' productivity or kills them, either by direct damage or by facilitating invasion by Phytophthora spp. and other root pathogens (Rogers et al. 1996, Stansly et al. 1997). However, field surveys of these and many other subterranean insect populations are rarely feasible because they involve labor-intensive, destructive excavation of root systems (Villani and Wright 1990).

A search for new tools led recently to development of a system that uses a soil probe with an accelerometer to detect subterranean sounds and a signal analysis program or experienced listener to discriminate insect sounds from background noise (Mankin et al. 2000a). We conducted tests in three different experimental citrus groves in Florida and Puerto Rico to consider the reliability of the new system in a variety of field environments. Acoustic activity detected at probes inserted into soil underneath citrus trees was recorded by a listener with headphones. The recordings were analyzed separately by digital signal pro-

Materials and Methods

Acoustic Measurements. The acoustic system included an accelerometer (sensitivity $10~\text{pC/ms}^{-2}$, weight 54~g), a charge amplifier, a digital audio tape recorder, headphones, and a digital signal processing system (Mankin 1994, Mankin et al. 2000a, 2000b). The accelerometer was attached to a 30-cm steel probe, pushed into the soil at an angle to pass near the crown of the citrus tree roots.

Depending on the level and complexity of background noise, we expected to detect larval activity within a 10- to 30-cm radius around the probe. Loud vehicular noise, talking, or wind >20 km/h can degrade signals sufficiently to obscure the insect generated sounds, but in low or moderate background noise, D. abbreviatus and Phyllophaga spp. are easily detected over 10–30 cm distances (Mankin et al. 2000a). We also expected to find differences in sound quality among the different recording locations because the transmission and attenuation of sound at different frequencies is strongly affected by differences in soil composition and packing (Liu and Nagel 1993, Mankin et al. 2000a).

Recording Procedures. An initial test was done with artificially infested citrus trees in a U.S. Department of Agriculture, Agricultural Research Service (USDA-ARS) experimental grove near Apopka, FL. Uninfested 1-yr-old Swingle, *Citrus paradisi Macf* × *Poncirus trifoliata* (L.), rootstock seedlings were placed in

cessing software. Organisms recovered by excavating the soil around the probes were categorized by pest status. The predicted likelihood of infestation was compared with the actual counts of insect pests and other organisms recovered at each recording side.

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a cleared area of the grove in sandy soil. Three weeks later, each tree was infested by digging a small hole at the base and adding five each of larvae weighing 25 ± 15 , 100 ± 15 , and 200 ± 15 mg, (approximate weights of fifth, sixth, and seventh instar, respectively, Quintela et al. 1998). It was not known in advance which age would be most likely to survive, and previous monitoring of larvae of different ages in the laboratory had found no significant sound pulse differences (Mankin et al. 2000a; R.W.M., unpublished data). Larvae were obtained from a laboratory colony maintained by the U.S. Horticultural Research Laboratory, Orlando, FL (Lapointe and Shapiro 1999).

Acoustic monitoring began on day 13 and testing was repeated on day 34. A >180-s period was recorded at each test site, and a listener with headphones subjectively rated the likelihood of pest infestation at the time of recording (see *Listener Analysis*). The recorded signals were later digitized and analyzed by a signal processing system in the laboratory (see *Computer Analysis*).

Preliminary analysis of the results suggested that the insect activity had declined between day 13 and 34. Consequently, we infested the trees again on day 37. After the second infestation, additional recordings were made on days 50, 54, 57, and 78. The trees were excavated on day 78 and any visible organisms were recovered from the roots.

The second and third tests were done using the same recording and listening procedures at contrasting sites in Puerto Rico, a D. abbreviatus homeland (Woodruff 1985). Test 2 primarily involved rootstock seedlings planted 1 yr earlier in mountainous clay soil at the Adjuntas Experiment Station of the University of Puerto Rico. Test 3 involved seedlings planted 1 yr earlier at Adjuntas, and seedlings planted in coastal lowlands at the Isabela Experiment Station. The trees were excavated and the roots were examined immediately after recording except for one large tree in test 2. This tree was several decades old and only the volume within ≈ 40 cm of the probe was excavated.

Categorization of Excavated Samples. Because D. abbreviatus and Phyllophaga spp. larval sound pulses strongly overlap in their temporal and spectral characteristics, they could not be reliably distinguished from each other by either the computer program or a listener with headphones (Mankin et al. 2000a). However, the rhythms and frequency patterns of sounds generated by nonpest earthworms, millipedes, slugs, ants, or adult ground beetles often were sufficiently different from these two pests to enable reliable discrimination. For purposes of this study, we therefore pooled the counts of *D. abbreviatus* and *Phyllophaga* larvae, categorizing them together as pest insects. We pooled into a nonpest category the counts of any nonpest organisms that could generate sounds interfering with detection of pest insects.

Listener Analysis. Listeners were trained to distinguish *D. abbreviatus* or *Phyllophaga* spp. sound pulses from sounds of above-ground insects, vertebrates, vehicles, and wind by practice with verified recordings and comparisons of spectral and temporal analyses of

insect sound pulses and background noises. The training included a 4- to 8-wk period using the accelerometer system in laboratory and field environments, listening to files of previously recorded sounds, and visual comparisons of differences in signal durations and spectra using the computer (Mankin et al. 2000a). Such training typically provides sufficient experience to identify important features of subterranean grub sound pulses and distinguish them from many background noises.

After recording and listening at each site in these experiments, the listener rated the likelihood of *D. abbreviatus* or *Phyllophaga* spp. pest infestation. The rating scale was: *low*, no subterranean sounds or only a few faint grub sound pulses, easily lost in the noise background; *medium*, sporadic or fairly continuous but faint grub sound pulses, sometimes obscured by background noises; *high*, frequent grub sound pulses with a high signal level, easily distinguished from background.

Computer and Statistical Analysis. The signals recorded on the digital audio tape recorder were digitized at 25 kHz and analyzed by author-written software (Mankin 1994; Mankin et al. 2000a, 2000b). Moving and feeding larvae generated short (2–5 ms) broadband pulses that were distinguished from many other sounds by computer subroutines that analyzed differences in temporal pattern or frequency.

The analysis of each digitized file began with a screen for periods where excessive background noise interfered with insect sound detection. These periods, excluded from further analysis, typically were short sections at the beginning and end of the file. In the remainder of each file, signals with peaks exceeding a user-adjustable minimum threshold were passed to an analysis subroutine for further processing. (The default minimum threshold was 20% of the root mean square +1% of the maximum signal level in the recording.) A spectrum was calculated for a 3-m segment (pulse) centered on each peak. The spectrum was compared with insect and background noise profiles derived from recordings where the soil organisms were verified by excavation. Different grub profiles were used for each grove because differences in the soil composition and packing caused different signal attenuation patterns at the different locations (Liu and Nagel 1993, Mankin et al. 2000a). This effect is more pronounced at higher frequencies than at low frequencies, because attenuation in all soils decreases as the frequency decreases. For the recordings at Apopka, insect sound profiles were constructed by averaging 512-point spectra from 17 to 233 pulses in three different relatively noise-free sections of recordings where only *D. abbreviatus* larvae were recovered. For the recordings at Adjuntas, a single insect sound profile was adequate for grub identification. It was constructed by averaging 49 sound pulses from an 82-s noise-free segment of a recording where only D. abbreviatus were present. The background noise profiles were average spectra of 193 and 267 noise pulses in sections of two recordings at Apopka where no soil organisms were present. Because the two noise profiles derived from the Apopka site successfully matched most background noises in recordings from all three sites, they were used for all the analyses. At these low frequencies (see above), the differences in the transmission characteristics of the soil at each site affected the spectral patterns less strongly than at the higher frequencies of the grub profiles.

In comparing a pulse with a grub or noise profile, the square of the difference between the pulse and profile spectrum level was calculated at each frequency point between 0.3 and 2 kHz. The squared differences were summed and divided by the total number of differences to obtain the pulse's average deviation from each profile. Each pulse was assigned to the profile with the smallest average deviation and was categorized as a grub pulse if it matched one of the grub sound profiles. However, if any single spectrum level difference exceeded a user-set single-level threshold. or if the smallest average deviation exceeded a user-set average-deviation threshold, the pulse was classified as unspecified noise. The settings for the averagedeviation threshold were determined primarily from previous experience with files from sites where only D. abbreviatus had been recovered (Mankin et al. 2000a). Specified and unspecified noise pulses were discarded from further analysis. If trains of otherwise valid grub pulses occurred in bursts >20 ms in duration, the signals also were discarded as potential noise. The use of >1 grub sound profile and >1 noise profile increased the overall reliability of comparisons across multiple recordings. Matches to one grub or one noise profile usually predominated in any single recording.

The activity rate was calculated as the number of grub pulses divided by the analysis period for each data file. The file was discarded if <60-s of the total recording period was usable. Regression analysis for numbers of organisms recovered on grub pulse activity rate was performed using PROC GLM (SAS Institute 1988).

Results and Discussion

At all three locations, larvae of *D. abbreviatus* and *Phyllophaga* spp. produced short (2–5 ms) grub pulses that were easily distinguished from most nonpest organisms and extraneous background noises. One example of the signals recorded from the Apopka grove is shown in Fig. 1A, and the spectral profiles used by the computer to distinguish grub pulses from background noises are shown in Fig. 1B. The grub pulse activity rates at different recording times at different sites and the numbers of organisms recovered from different sites in the Apopka grove are summarized in Table 1, along with the listener ratings on the day the trees were excavated.

The mean activity rates at Apopka varied considerably by both tree and by test date. A conspicuous decline in the mean activity rates from 24 to 7 grub pulses · min⁻¹ occurred between days 13 and 34 (Table 1) which resulted in a second infestation treatment on day 37. One factor that may have contributed to the observed decline was an abnormally high soil temper-

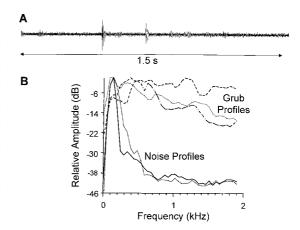


Fig. 1. (A) 1.5-s sample oscillogram of sound pulses recorded from a *D. abbreviatus* larva in soil. (B) Examples of three *D. abbreviatus* (solid, dot, and dash lines) and two background noise (dot-dash, and small-dash) spectral profiles used by signal analysis program to classify sounds from Florida sites as grub pulses or noise pulses.

ature during the first weeks after infestation (36°C on day 13 and 34°C on day 34). Such temperatures are well above optimal larval growth temperatures of 26–30°C (Lapointe 2000).

The grub pulse activity rates and the actual infestation levels were higher in test 2 at Adjuntas (Table 2) than at Apopka. The listener rated all 10 trees at high likelihood of infestation. The mean activity rate for all trees was 24 grub pulses · min ⁻¹. The lowest grub pulse activity rates, the lowest listener ratings of the likelihood of pest infestation, and the lowest actual infestation levels occurred in the final test at Isabela and Adjuntas (Table 3) where only five of 20 sites were infested. The computer-rated analysis of grub pulse activity rates is not shown in Table 3 because 20–35 km/h winds confounded analysis of recordings at Isabela.

Predicted Versus Actual Infestations. The primary goal of the study was to consider whether computer analyses or the judgment of a listener with headphones could predict infested sites and, if so, whether they could estimate the size or the vigor of the infestation. Reductions in larval activity after a pesticide treatment, for example, might provide a quick estimate of the treatment's efficacy. The results in Tables 1–3 suggest that the system performs well in predicting infestations, and in some cases, it may be possible to use it in a sampling scheme to track average changes in infestation levels in a group of trees over time.

In general, the highest rates of activity on the day of excavation and the highest mean rates of activity were associated with trees where larval pests were recovered. At 25 of 30 sites, the listener ratings correlated correctly with the presence and absence of infestation (i.e., correlating *medium* or *high* with infested, and *low* with uninfested). The activity rates and listener ratings were higher for the Adjuntas (Table 2) than the Apopka site, and correspondingly greater numbers of

Table 1. Sound pulse activities under citrus trees at Apopka, FL, on different days after two treatments with *D. abbreviatus larvae* (day 0 and day 37), compared with the numbers of organisms excavated, and listener rating of pest infestation likelihood

Tree ^a no.	Activ	Activity rate (grub pulses·min ⁻¹) recorded on day after 1st (2nd) larval treatment					Listener- rated	No. pest insects ^b	Mean ^c grub
	13	34	50(13)	54(17)	57(20)	78(41)	infestation likelihood	counted	pulses • min ^{−1}
11	1	2	15	151	5.0^{e}	_	Medium	1	57
2	226	3	25	28	32	30.4^{e}	Medium	2	29
15	1	8	16	76	15	2.1^{e}	Medium	1	27
1	1	2	81	3	4	12.4^{e}	Medium	1	25
3	35	1	15	44	37	0.3^{e}	Medium	1	24
5	2	1	28	6	15	0.5^{e}	High	1	13
20	12	2	8	9	20	4.0^e	Medium	1	10
7	33	10	-d	25	13	0.0^e	Medium	0	10
8	1	16	3	12	22	0.0^{e}	Low	0	9
4	33	5	6	0	19	5.2^{e}	Low	2	8
16	0	7	11	7	12	0.7^{e}	Low	0	8
10	24	48	2	1	18	3.3^{e}	Low	1	6
6	-d	13	0	11	7	0.0^{e}	Medium	1	5
9	-d	7	7	7	6	1.4^e	Low	0	5
18	14	2	3	7	6	2.2^{e}	Medium	0	5
12	2	7	2	8	4	0.0^{e}	Medium	1	4
14	17	2	2	2	10	0.0^{e}	Low	0	4
13	1	1	3	0	3	3.2^{e}	Medium	0	2
17	-d	2	2	4	1	2.0^e	Low	0	2
19	2	3	3	1	2	0.0^e	Low	0	2
$Mean^f$	24	7	12	20	13	3			

^a Trees are sorted by descending levels of mean rate for recordings after second treatment.

D. abbreviatus or Phyllophaga larvae were recovered. At the Adjuntas and Isabela sites (Tables 2–3) the listener ratings also correlated well with the presence and absence of D. abbreviatus and Phyllophaga larval pests. Except at tree A1 in Table 3, the listener correctly predicted when D. abbreviatus was not present at sites where other nonpest organisms (including earthworms, slugs, millipedes, centipedes, a mouse, and a Coquí tree frog) were recovered.

To simplify the use of the acoustic system as a decision-making tool, we considered how the activity

Table 2. Sound pulse activities under citrus trees at Adjuntas, PR, compared with numbers of organisms excavated from the root systems

Tree no.	Grub pulses · min ⁻¹	No. pest ^a organisms counted	No. nonpest ^b organisms counted
21	31.7	10	0
27	29.3	4	4
28	28.0	4	2
22	28.0	8	4
29	27.3	16	5
26^c	27.0	2	0
24	25.0	7	3
25	21.7	12	0
23	16.3	5	0
30	2.3	6	0

^a D. abbreviatus, and Phyllophaga spp.

rate might be used to estimate the likelihood that a recording site was infested. We pooled and sorted the results for recordings in Tables 1 and 2 where the roots were examined immediately after testing to derive thresholds for minimum activity rates at infested sites and maximum rates at uninfested sites. All sites with rates >0 grub pulses · min⁻¹ (9 trees, numbers 2, 21, 22, and 24-29) had root systems containing D. abbreviatus or Phyllophaga larvae. Seven of 11 sites with rates ≤2 grub pulses · min⁻¹ had no organisms recovered (numbers 7-9, 14, 16, 17, and 19). This suggested that the recording sites could be rated as having a high (>20), medium $(\le 20 \text{ and } > 2)$, or low $(\le 2 \text{ grub pulses})$ ·min⁻¹) likelihood of infestation. The resultant ratings (Table 4) corresponded well with the observed infestation levels, as did the subjective ratings of listeners in all three tests. The relationship between computer-rated likelihood and the observed infestation was highly significant ($\chi^2 = 10.26$, df = 2, P < 0.01), as was the relationship between listener-rated likelihood and the observed infestation ($\chi^2 = 22.53$, df = 2, P < 0.005). There also was a highly significant correspondence between the computer and the listener ratings, as shown in Table 5, which compares ratings for all 115 recordings in the FL grove ($\chi^2 = 26.6$, df = 4, P < 0.005).

Some of the differences between the computer and listener ratings undoubtedly are due to the greater capability of an experienced listener to distinguish pest insect larvae from background and nonpest noises. However, the computer ratings correlate bet-

 $[^]b\,D.$ abbreviatus or Phyllophaga spp., except one wireworm (Coleoptera: Elateridae) at Tree 10.

 $[^]c$ Mean rate for days 50(13) B 78(41) after first and second treatment.

 $^{^{\}it d}$ 20–35 km/h winds confounded computer analysis of these recordings.

^e Activity rates for recordings made just before excavation.

f Mean rate for all trees on given day.

^b Earthworms (Lumbricidae spp.), slugs (Stylommatophora spp.), millipedes (Diplopoda spp.), and centipedes (Chilopoda spp.).

^c Due to the large size of the tree only the immediate area around the spike was excavated.

Table 3. Listener-rated infestation likelihoods and numbers of organisms sifted from citrus root systems immediately after recording in test 3 in groves at Isabela (I1-I14) and Adjuntas (A1-A6), PR

Tree no.	Listener-rated infestation likelihood	No. pest ^a organisms counted	No. nonpest ^b organisms counted
I3	Medium	2	4
A4	Medium	1	4
I 9	Low	1	1
I4	Low	1	1
I1	Low	1	0
A 1	Medium	0	5
I 7	Low	0	6
I13	Low	0	5
A6	Low	0	4
A 2	Low	0	3
A5	Low	0	3
I2	Low	0	2
I5	Low	0	1
I8	Low	0	1
I12	Low	0	1
I10	Low	0	0
I11	Low	0	0
I14	Low	0	0
A 3	Low	0	0
I 6	Low	0	0

^a D. abbreviatus, and Phyllophaga spp.

ter with the listener ratings than with the actual counts of pest larvae ($\chi^2 = 26$ versus 10), possibly because the larvae do not generate sounds continuously. Both the computer and listener ratings were most reliable when a high rate of activity was detected (9/9 correct predictions of pest infestation in the computer ratings and 11/11 in the listener ratings). They were least reliable when negligible activity was detected (7/11 correct predictions of uninfested trees in the computer ratings and 20/25 correct predictions in the listener ratings), but still better than chance. Preliminary studies with D. abbreviatus in the laboratory indicate that larvae are active in \approx 70% of 180-s monitoring sessions, with sporadic bouts of inactivity. This suggests that the reliability of an acoustic monitoring plan could be maximized by use of a sequential sampling procedure (e.g., Brewer et al. 1994), increasing the monitoring period when the activity rate is low.

Prediction of Infestation Level from Sound Activity. A third goal of the study was to determine whether the numbers of organisms at different recording sites could be predicted from an easily measured characteristic of the recording. We examined the relationship between activity rate and the number of pest organisms using the pooled results for sites where the trees were examined immediately after recording. Regression analysis (SAS Institute 1988) was applied to both the original and logarithmically (base 10) transformed data. The line of best fit (Fig. 2) was the equation

Log (no. organisms + 1) =

Table 4. Numbers of uninfested and infested sites rated at different likelihoods of pest infestation by computer analysis and listener judgement in tests 1-3 where site was excavated immediately after recording

D. i. l	Compute	r-rated	Listener-rated		
Rated likelihood	No. uninfested sites	No. infested sites	No. uninfested sites	No. infested sites	
Low	7	4	20	5	
Medium	2	8	4	10	
High	0	9	0	11	

Basis of computer-rated likelihood: low, activity rate ≤ 2 ; medium, $20 \leq$ activity rate > 2; high, activity rate > 20 grub pulses \cdot min $^{-1}$. Basis of listener-rated likelihood, see *Materials and Methods*.

$$A + B Log (activity rate + 1), [1]$$

where $A = 0.028 \pm 0.08$ standard error (SE) is the intercept, and $B = 0.57 \pm 0.08$ SE is the slope. A and B have no units, and the activity rate is in units of pulses · min⁻¹. The root mean square error was 0.26 and the coefficient of determination, r^2 , was 0.62.

Though significant, the relationship between the activity rate and the number of organisms present at a recording site was not as strong as the relationship between activity rate and the likelihood of infestation. This may be due partly to the sound-insulating properties of soil. Even when 10–20 insects were associated with the root system, only a few were within 20–30 cm of the accelerometer probe. Insects further away are not as likely to be detected because their acoustic signals attenuate rapidly with distance (Mankin et al. 2000a). Consequently, the likelihood of detection is increased if a scout has knowledge of the pest insect behavior and places the probes close to expected sites of activity.

Potential Applications. Acoustic monitoring techniques show promise as survey tools for rapid detection of citrus root weevils, white grubs, and other subterranean insect pests in field environments (see also Mankin et al. 2000a). The system in this study can be used in a variety of field environments at least as rapidly as destructive excavation techniques. However, the system needs continued development to meet other urgent needs of soil-insect entomologists, including methods for estimating population numbers and reliable discrimination of pest species from nonpest organisms. For future improvement of population estimates, we are considering the possibility of counting the number of different sound-producing locations

Table 5. Comparison between computer ratings and listener ratings of the likelihood of infestation at recording sites on different days in the Apopka, FL, grove

Computer-		Listener-rated	
rated	Low	Medium	High
Low	25	14	0
Medium	19	34	3
High	4	10	6

Each site was categorized according to both the computer ratings and the listener ratings listed in Table 4.

^b Earthworms (Lumbricidae spp.), slugs (Stylommatophora spp.), millipedes (Diplopoda spp.), and centipedes (Chilopoda spp.), one mouse (*Mus musculus*) (tree 17), 1 Coqui tree frog (*Eleutherodactylus* spp.) (tree A4).

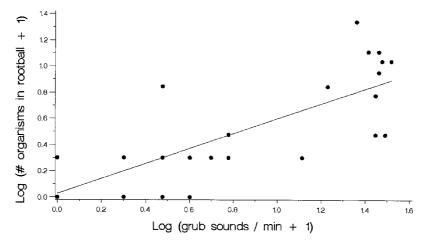


Fig. 2. Relationship between the number of pest organisms present in the citrus tree root systems and the rate of grub pulse activity detected.

as has been done successfully with stored-product insects (Shuman et al. 1993, 1997, Mankin et al. 1997, Weaver et al. 1997). Long-term, a preferred solution is to completely automate the identification process, eliminating the need for experienced listeners.

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